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Research Article

Vegetative Propagation of *Berlinia grandiflora* by Leafy Stem Cutting in the Guinean Savannah Highlands of Cameroon

^{1,2}Kouojip Nganjouong Jacquino, ³Oumarou Zéphirin, ¹Dangai Youhana, ²Zang Priscille, ¹Loura Benoît and ²Mapongmetsem Pierre Marie

¹Department of Biological Sciences, Faculty of Sciences, University of Maroua, P.O. Box 46, Maroua, Cameroon

²Laboratory of Biodiversity and Sustainable Development, Faculty of Sciences, University of Ngaoundéré P.O. Box 454, Ngaoundéré, Cameroon

³Department of Plant Sciences, Faculty of Sciences, University of Bamili, P.O. Box 39, Bamenda, Cameroon

Abstract

Background and Objective: *Berlinia grandiflora* is a wild, non-domesticated woody species of high socio-economic value. Its population dynamics are regressive due to anthropogenic and natural pressures. To domesticate these species, vegetative propagation by stem cuttings was carried out in the Guinean Savannah Highlands of Cameroon. **Materials and Methods:** The uninodal leafy stem cuttings 5 cm in length came from rejuvenated adult trees. The experimental design used was a split-plot with 3 replications, 480 cuttings were handled. The parameters studied in a polypropagator were the substrate effect (black soil, sand/sawdust, black soil/sawdust and sawdust) and the node position effect. The statistical data collected were analyzed using Statgraphics Plus 5.0. and the Duncan's Multiple Range test to compare the means. **Results:** The substrate influences root formation and had no influence on the number and length of roots formed. Black soil/sawdust was the most favorable substrate ($34.16 \pm 19.75\%$ of cuttings rooted). The black soil induced the greatest number of roots per cutting (1.29 ± 0.56) as well as sand/sawdust for the length of roots (11.90 ± 9.31 cm). The node's position did not affect rhizogenesis, the number or even the length of roots formed. However, numerically, node N3 gives the best results ($28.33 \pm 16.96\%$ of cutting rooted, 1.37 ± 0.59 roots/cutting and roots of 12.35 ± 6.97 cm long). **Conclusion:** This study opens interesting prospects for the multiplication and domestication of *B. grandiflora* by vegetative propagation and its integration into existing peasant production systems.

Key words: *Berlinia grandiflora*, stem cuttings, substrate, node position, Guinean Savannah highlands

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Corresponding Author: Kouojip Nganjouong Jacquino, Faculty of Sciences, University of Maroua, P.O. Box 46, Maroua, Cameroon
Tel: (+237) 696.320.175/(+237) 679.259.997

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Berlinia grandiflora is a plant of great socio-economic interest in the Guinean Savannah Highlands (GSH) in Cameroon, better known as "Djin" in fufuldé¹. This species belongs to the Fabaceae family, confined to Tropical Africa². *Berlinia grandiflora* is a woody plant that lives in forest galleries and forest edges in Sudanian and Guinean zones, on deep, well-drained soils³. The leaves and bark are used in traditional medicine to treat fibroids⁴. A decoction of the leaves is used as a febrifuge and antiemetic⁵. Stem bark extract has analgesic activity⁶. Bark extracts have shown appreciable antimicrobial and antihelmintic properties⁷. Betulinic acid extracted from the trunk bark is an inhibitor of HIV reproduction, an antimalarial, anti-inflammatory, anthelmintic and antioxidant properties⁸. According to Ouattara *et al.*⁹, Djin is used as fuelwood. The pods are used to make pot-cleaning nets. The *B. grandiflora* wood is suitable for interior trim, shipbuilding, vehicle bodies, furniture, railway sleepers, poles and plywood¹⁰. In Cameroon, the seeds are consumed as beans in the Tikar plain and by some Nigerian communities. *Berlinia grandiflora* makes a significant contribution to improve the incomes of farming households. In 2008, for example, the average price of a 100 kg bag of *Berlinia grandiflora* seeds on the local market was \$175.82-197.80, resold in Nigeria at an average value of \$549.45-659.34¹. These seeds are highly sought after by Nigerian traders who crisscross the villages of Adamawa-Cameroon to collect them. Given the socio-economic importance of this species, it remains in the wild. It is subject to strong anthropogenic and natural pressures, as systematic seed collection and bushfires prevent the plant from regenerating and have a significant effect on population dynamics¹¹. Moreover, as *B. grandiflora* is a forest gallery plant, its natural regeneration is compromised by its seeds, which sometimes fall into the water and rot or are washed away¹². This woody species is marked by the rarefaction or absence of young individuals and consequently has a regressive dynamic. It deserves not only to be protected in the wild but also to be domesticated with a view to its introduction into existing farming systems. What's more, little is known about the propagating techniques of *Berlinia grandiflora*.

Many tropical species of nutritional or commercial value regenerate in ways that are not well understood¹³. Vegetative propagation is the most important method used for agroforestry species¹⁴. Of the many vegetative propagation techniques used in agroforestry (grafting, cuttings, layering and suckering), stem cutting in a polypropagator is less costly

and requires less technology¹⁵. The lack of knowledge about the multiplication of this species in the GSH justifies and motivates the present study. The present work aims to determine the effect of substrate and node position on the rooting ability of leafy stem cuttings of *B. grandiflora*.

MATERIALS AND METHODS

Study site: The cuttings experiment took place at the nursery of the Laboratory of Biodiversity and Sustainable Development located in Manwi near the Bini River (Alt: 1079 m, 7°24'51"N and 13°32'58"E) and the plant material came from adult trees rejuvenated in the forest galleries of Mayo Djarandi (Alt: 1010 m, 7°29'33.6"N and 13°16'12.2"E). Manwi and Mayo Djarandi are located in the Guinean Savannah Highlands (GSH) of the Adamawa Region of Cameroon. The GSH is located between the Sudano-Sahelian North and the humid forest South. The climate is a humid Sudano-Guinean type. This climate has two seasons: A rainy season from April to October and a dry season from November to March, with mean annual temperatures and rainfall of 21.6°C and 1884.1 mm, respectively in 2021 (ASECNA-Ngaoundere Weather Observation Office 2023). The relief is uneven and the soil in the region has a high aluminium and iron content¹⁶.

Experiment: The trial consisted of evaluating the effect of substrate and node position. The cuttings were taken from rejuvenated adult trees. The rejuvenation operation consisted of felling 16 healthy adult trees, 25 cm above the ground¹⁷. The trees were felled on July 30, 2020. After reiteration of the stumps, the orthotropic shoots bearing at least 5 nodes (Fig. 1) were removed using pruning shears on April 06, 2021.

The cuttings were taken in the morning before periods of high heat to limit their dehydration¹⁸. This operation ensured that the cells remained turgid. These cuttings were protected by moistened newspaper and transported to the nursery using a cooler containing ice blocks for immediate planting¹⁹.

Once in the shed at the nursery, uninodal sections 5 cm long were taken using pruning shears¹. These stem segment cuttings were classified according to the position of the nodes; basal (N1), apical (N4), median close to N1 (N2) and median close to N4 (N3). During this operation, precautions were taken to avoid damaging the base of the cuttings or crushing the cells. The leaves were cut in half to reduce the leaf area which could limit evapotranspiration.



Fig. 1: Rejuvenated *Berlinia grandiflora* trunk

The cuttings prepared in this way were grown in 4 substrates previously prepared in a polypropagator. These substrates were: Black soil (Tn), sand/sawdust (Sa/Sc), black soil/sawdust (Tn/Sc) and sawdust (Sc). These cuttings were pricked in a two-thirds vertical position. The trial ran from April 06, 2021, to October 05, 2021. The cuttings were watered each day in the morning using a sprayer that discharges fine droplets of water. Assessments were carried out monthly until the end of the trial. This operation consisted of carefully loosening a cutting (without breaking the roots that had formed), observing its base and then, putting it back in place. A cutting is said to be rooted if the length of the root is greater than or equal to 1 cm²⁰.

The experimental design used was a split-plot with 3 replications in which the type of substrate was the main treatment with four variants and the secondary treatment consisted of the position of the nodes with four variables. The experimental unit consisted of 10 cuttings. A total of 480 cuttings were handled (4 media × 4 nodes position × 10 cuttings × 3 replications).

Statistical analysis: Data analysis concerns variance. Significant means were separated using the Duncan's Multiple Range test. The statistical program used for variance analysis was Statgraphics plus 5.0 at the 0.05 level of probability. Microsoft Word 2010 Excel spreadsheet was used for data entry and graphing.

RESULTS

In this trial, there was no leaf bud formation and therefore no new leaves. The leaves of the cuttings still intact remained functional for the formation of missing organs such as roots. The evaluation of rhizogenesis-related parameters such as the number of cuttings that formed roots, the number of roots per cutting and the length of these roots was assessed up to the 26th week after the trial was set up, i.e., 6 months. Before the roots emerge, calluses form (Fig. 2).

After the trial was set up, it took 6 weeks for roots to appear on some cuttings (Fig. 3). This is the root emergence lag time.

Rooting rate

Effect of substrate on root formation of stem cuttings:

From the second month, rooting was regular in all substrates and progressed in the same direction until the end of the trial (Fig. 4).

At the end of the trial (after 26 weeks), the rooting percentage varied from $15.83 \pm 15.05\%$ in the sand/sawdust mixture to $34.16 \pm 19.75\%$ in the black soil/sawdust mixture (Table 1). This variation is confirmed by the fact that there is a significant difference between substrates ($0.0486 < 0.05$).

Effect of node position on root formation of stem cuttings:

At the end of the trial, the rooting percentages varied from $15.83 \pm 11.64\%$ for the proximal node (N1) to $28.33 \pm 16.96\%$ for N3 (median close to N4) (Table 2). This difference is only apparent since the analysis of variance shows no significant difference between nodes ($0.2448 > 0.05$).

Effect of node position × substrate interaction on rooting of stem cuttings:

The rooting rate of stem cuttings ranged from $6.66 \pm 11.54\%$ for N4 cuttings placed in the sand/sawdust medium to $46.66 \pm 20.81\%$ for N4 cuttings placed in the black soil/sawdust medium. This difference is not established as there is no significant difference between this interaction ($0.2412 > 0.05$).

Number of neoformed roots per stem cutting

Effect of substrate on the number of neoformed roots per stem cutting:

The number of neoformed roots increases steadily in all substrates, with a numerical superiority in substrates made of black soil/sawdust and black soil substrate (Fig. 5). The average number of roots per cutting varied from 1.07 ± 0.84 in the sawdust-based growing medium to 1.29 ± 0.56 in the black soil substrate, compared with an overall average of 1.17 ± 0.63 (Table 1). There was no significant difference between the four substrates ($0.6309 > 0.05$).



Fig. 2: Callus formed on a leafy *Berlinia grandiflora* cutting



Fig. 3: Uninodal cuttings of *Berlinia grandiflora* with five adventitious roots

Table 1: Root parameters according to substrate after 26 weeks

Rooting parameters	Substrates				Total
	Sand-sawdust mixture	Sawdust	Black soil	Blacksoil-sawdust	
Rooting percentage	15.83±15.05 ^a	22.50±16.58 ^{ab}	21.66±13.37 ^{ab}	34.16±19.75 ^b	23.54±16.18
Number of roots	1.07±0.84	1.04±0.53	1.29±0.56	1.28±0.57	1.17±0.63
Root length (cm)	11.90±9.31	8.48±6.05	11.42±5.7	10.29±4.11	10.52±6.30

Means followed by the same letters are not statistically different ($p>0.05$)

Table 2: Root parameters following node after 26 weeks

Rooting parameters	Nodes position				Total
	N1	N2	N3	N4	
Rooting percentage	15.83±11.64	25.83±15.64	28.33±16.96	24.16±22.34	23.54±16.64
Number of roots	1.00±0.70	1.29±0.49	1.37±0.59	1.02±0.70	1.17±0.63
Root length (cm)	9.76±7.78	12.25±5.51	12.35±6.97	7.72±5.00	10.52±6.31

N1: Proximal node, N2: Middle node close to N1, N3: Middle node close to N4 and N4: Distal node

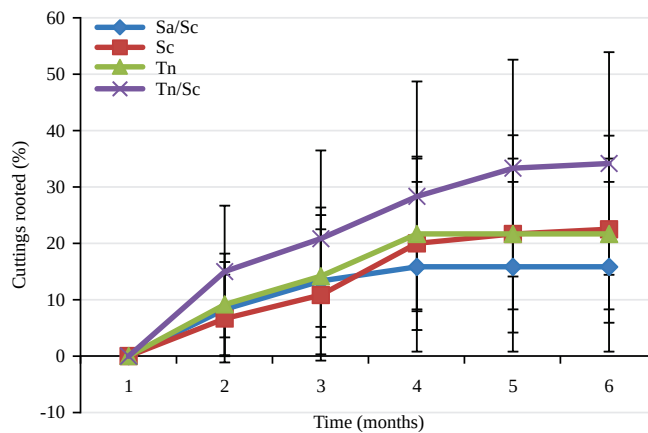


Fig. 4: Percentage of rooting of uninodal *Berlinia grandiflora* cuttings according to substrate
Sa/Sc: Sand/sawdust mixture, Sc: Sawdust; Tn: Black soil and Tn/Sc: Black soil/sawdust mixture

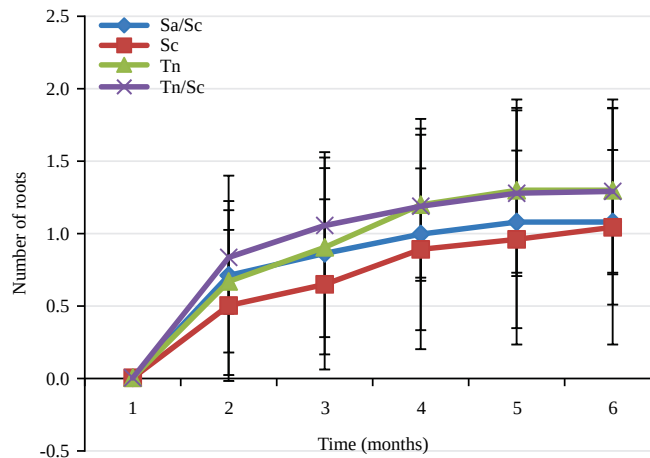


Fig. 5: Number of roots per cutting of *Berlinia grandiflora* stem segments following substrates
Sa/Sc: Sand/sawdust mixture, Sc: Sawdust, Tn: Black soil and Tn/Sc: Black soil/sawdust mixture

Effect of node position on the number of neoformed roots per stem cutting:

In terms of the number of roots per cutting, position N3 appears to be the best performing since it recorded the highest number of roots per cutting (1.37 ± 0.59) (Table 2). In contrast, node N1 (1.00 ± 0.70) induced the lowest number of roots per cutting. The variability observed between the different node positions was not established, since the analysis of variance revealed the existence of a non-significant difference between the nodes ($0.3448 > 0.05$).

Effect of node position $\times$ substrate interaction on the number of neoformed roots:

The node position $\times$ substrate interaction was not significant on the rooting rate on the number of roots per stem cutting ($0.2321 > 0.05$). The number of roots formed is not influenced by any cuttings with nodes in any position placed in a substrate.

Length of neoformed roots per stem cutting

Effect of substrate on neoformed root length per stem cutting:

The length of *B. grandiflora*'s neoformed roots showed steady growth over the 6 months of observation (Fig. 6). The substrate made from a mixture of sand and sawdust performed best, with roots 11.90 ± 9.31 cm long (Table 1). However, there was no significant difference between the different substrates ($0.6138 > 0.05$).

Effect of node positions on neoformed root length per stem cutting:

Root length ranged from 7.72 ± 5.00 cm for N4 node cuttings to 12.35 ± 6.97 cm for N3 node cuttings (Table 2). This variation is not also established because the analysis of variances shows a non-significant difference between the nodes ($0.2925 > 0.05$).

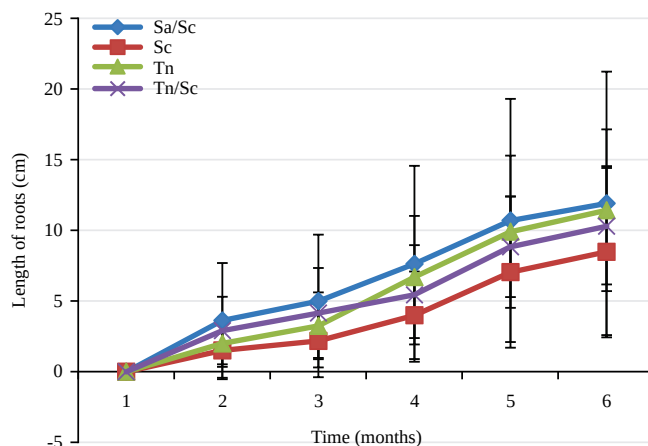


Fig. 6: Length of roots per cutting of *Berlinia grandiflora* stem segments according to substrates

Effect of substrate×node position interaction on the size of neoformed roots per stem cutting: In terms of root length, there was no significant difference in the node position×substrate interaction ($0.7371 > 0.05$). Nevertheless, the best interaction (17.13 ± 3.58) is observed between node N2×sand/sawdust mix and the worst interaction (4.33 ± 7.50) between node N4×sand/sawdust mix.

DISCUSSION

Rooting rate: The root emergence lag time in *Berlinia grandiflora* is 6 weeks. This time is short compared with that reported by Atangana *et al.*²¹ on a clone variant of *Allanblackia floribunda*, which is 10 weeks. This 6 week root emergence period indicates that *Berlinia grandiflora* is suitable for stem cutting, since according to Leakey *et al.*²², most uninodal leafy stem cuttings on many tropical trees root after 6-8 weeks. Beyond this period, the plant is considered recalcitrant²³. All stem cutting took root after a bead of scar cells or callus had formed at the tip of the cutting. This reconstitution involves the functioning of new meristems, which did not pre-exist in the initial fragment and these new meristems result from cellular dedifferentiation²⁴. The callus generates root cells. In this trial, no cuttings that had formed a callus produced roots. The appearance of roots is earlier on some cuttings than on others because certain clones or varieties have a greater ability to root than others of the same species. *Berlinia grandiflora* is suitable for taking cuttings from stem segments and the different substrates used in the present work influence root formation. These observations corroborated those obtained by Tchoundjeu *et al.*²⁵, whose rooting media consisting of sawdust, sand and a homogeneous sand/sawdust mixture had a significant impact on *Prunus africana* rooting. Conversely,

substrates consisting of sand, decomposed sawdust and the sand/sawdust mixture did not affect the rooting of the *Dacryodes edulis* species²⁶.

The importance of the growing medium for the rooting of leafy stem cuttings is widely recognised^{15,27}. The quality of the substrate is a very important parameter for the success of the rooting process. The requirements of species about different substrates depend, among other things, on the effects of the substrate on water exchange with the cutting²⁸. In the present study, the nature of the substrate had an impact on rhizogenesis and the black soil/sawdust mixture proved to be the best rooting medium for *B. grandiflora* ($34.16 \pm 19.75\%$). This finding was also confirmed for *Vitex doniana* by Marie *et al.*¹. The advantage of the black soil/sawdust mixture over other substrates is that it has a high capacity to retain water and oxygen available to the cuttings. It is a light, porous substrate. The quality of this black soil/sawdust mixture substrate suggests that the species that root best in this type of substrate would be adapted to conditions of water saturation¹. This is the case for *B. grandiflora*, whose preferred ecological environment is the forest gallery.

The position of the nodes did not influence the rhizogenesis of stem segment cuttings of *B. grandiflora*. On the other hand, the position of the nodes had a significant effect on the rooting of cutting of species such as *Tetrapleura tetraptera* and *Vitex doniana*. According to the work of Marie *et al.*¹ and Olaniyi *et al.*²⁹, the best performance was observed with the basal node in *Tetrapleura tetraptera* and the apical node in *Vitex doniana*, respectively. Compared with the results of these authors, the rooting of cuttings varies according to the position of the node and the best position for rooting is problematic. Although the position of the nodes

does not influence root formation in the present work, it is clear that the rooting rate increases from the base to the apex of the stem. Marie *et al.*¹ reported that in the species *Vitex doniana*, the percentage of rooted cuttings decreases from the apical nodes to the basal nodes, as observed in the present work. This variation suggested the existence of a rooting gradient along a *B. grandiflora* stump shoot. The existence of this gradient would be the result, on the one hand, of inequality in the distribution of carbohydrates, minerals and hormones in the cuttings and on the other hand, of the diameter of the cuttings, which is greater in the basal nodes and, above all, of the totipotency of the apical nodes. The slight reduction in rooting observed in cuttings from node N4 is due to the vulnerability of these cuttings. The cuttings from this position are still made up of fragile cells, incapable of resisting trauma.

Number of neoformed roots per stem cutting: In *B. grandiflora*, the average number of roots per cutting in all substrates is 1.17 ± 0.63 . This number is identical to that obtained for *Pentaclethra macrophylla*³⁰. However, this result differs from those obtained for *Vitex doniana*, *Pausinystalia yohimbe* and *Irvingia gabonensis*, respectively, with an average number of roots of 4.26¹, 2.4³¹ and 1.8-7.8³². The number of roots per cutting in *B. grandiflora* was certainly not influenced by the characteristics of the substrates (porosity, pH, water and oxygen availability). The similarity of substrate response in relation to root number was also reported by Atangana *et al.*²¹ on a clone variant of *Allanblackia floribunda* and Tsobeng *et al.*³⁰ on *Pentaclethra macrophylla*. Although the suitability of the substrates was identical, black soil followed by the black soil/sawdust mix was better in terms of the ability to induce roots.

Length of neoformed roots: Root size was uniform regardless of the substrate used to grow *B. grandiflora* stem segment cuttings. This was also observed by Marie *et al.*¹ on *Vitex doniana* where the best substrate for root elongation was the black soil/sand mixture (6.1 cm). Compared with other studies, at 6 months, the average root length of cutting of *B. grandiflora* is relatively long (10.52 cm) compared with 5.96 cm on *V. doniana*, for example research by Marie *et al.*¹.

The position of the nodes on the stems does not influence root length. However, the best result was observed with node N3 (nodes close to the apex). This information is contrary to that obtained by Olaniyi *et al.*²⁹ on *Tetrapleura tetraptera* where the basal position (N1) showed the best root length (2.8 cm). The longest roots observed in the N3 nodes of the

cuttings of *B. grandiflora*, confirm the idea that the interesting characteristics of the apical zone, such as totipotency and richness in carbohydrates and enzymes, give them a good aptitude for developing roots.

The combination of the chemical composition of the substrate and the position of the nodes does not influence the rooting of stem cuttings. Although there was no significant difference in this interaction, it appears that the best interaction was obtained with the black soil/sawdust substrate and node N4, which, as noted above, were the best parameters.

CONCLUSION

This study shows that *Berlinia grandiflora*, a species with high socio-economic potential, is suitable for vegetative propagation by leafy stem-cutting segments. The substrate was the only parameter that influenced the rooting percentage of the cuttings and the black soil/sawdust substrate performed best. This study opens up interesting prospects for the multiplication and domestication of *B. grandiflora* by vegetative propagation and its integration into existing farming systems. The success rate obtained could certainly be improved. To achieve this, it would be necessary to carry out further studies on the influence of stem diameter and leaf area.

SIGNIFICANCE STATEMENT

Berlinia grandiflora is a wild woody species, that has not been domesticated yet and has a very high socio-economic value. Its population dynamics are regressive due to anthropogenic and natural pressures. The study aimed to evaluate the effect of substrate and node position on the rooting of leafy stem cuttings. The substrate influences root formation. Black soil/sawdust was the most favorable substrate. The black soil induced the greatest number of roots per cutting as well as sand/sawdust for the length of roots. The node's position did not affect rhizogenesis, the number or even the length of roots formed. This study opens interesting prospects for the multiplication and domestication of *B. grandiflora*.

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